Morlock MA, Schilder J, van Hardenbroek M, Szidat S, Wooller MJ, Heiri O.
Seasonality of cladoceran and bryozoan resting stage $\delta^{13}C$ values and implications for their use as palaeolimnological indicators of lacustrine carbon cycle dynamics. ***Journal of Paleolimnology*** **2017**

Seasonality of cladoceran and bryozoan resting stage $\delta^{13}C$ values and implications for their use as palaeolimnological indicators of lacustrine carbon cycle dynamics

MA Morlock[1,*], J Schilder[1], M van Hardenbroek[1,2], S Szidat[3], MJ Wooller[4] & O Heiri[1]

[1]Institute of Plant Sciences & Oeschger Centre for Climate Change Research, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland

[2]Geography and Environment, University of Southampton, Southampton SO17 1BJ, UK

[3]Department of Chemistry and Biochemistry & Oeschger Centre for Climate Change Research, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

[4]Alaska Stable Isotope Facility, University of Alaska Fairbanks, Fairbanks, Alaska, 99775, USA

* Corresponding author: marina.morlock@geo.unibe.ch

Key words Methane, *Daphnia*, Stable carbon isotopes, Invertebrates, Bryozoa, Cladocera

Abstract

The stable carbon isotope composition, expressed as $\delta^{13}C$ values, of chitinous resting stages of planktivorous invertebrates can provide information on past changes in carbon cycling in lakes. For example, the $\delta^{13}C$ values of cladoceran ephippia and bryozoan statoblasts have been used to estimate the past contribution of methane-derived carbon to lake food webs and variations in the $\delta^{13}C$ value of planktonic algae. Limited information, however, is available concerning seasonal variations in $\delta^{13}C$ values of these organisms and their resting stages. We measured the seasonal variation in $\delta^{13}C$ values of *Daphnia* (Branchiopoda: Cladocera: Daphniidae) and their floating ephippia over a 2-year period in small, dimictic Lake Gerzensee, Switzerland. Floating ephippia of *Ceriodaphnia* (Branchiopoda: Cladocera: Daphniidae) and statoblasts of *Plumatella* (Phylactolaemata: Plumatellida: Plumatellidae) were analysed during parts of this period. Furthermore, $\delta^{13}C$ values of remains from all three organism groups were analysed in a 62-cm-long sediment core. Throughout the year, *Daphnia* $\delta^{13}C$ values tracked the $\delta^{13}C$ values of particulate organic matter (POM), but were more negative than POM, indicating that *Daphnia* also utilize a relatively ^{13}C-depleted carbon source. *Daphnia* ephippia $\delta^{13}C$ values did not show any pronounced seasonal variation, suggesting that they are produced batch-wise in autumn and/or spring and float for several months. In contrast, $\delta^{13}C$ values of *Ceriodaphnia* ephippia and *Plumatella* statoblasts followed variations in $\delta^{13}C_{POM}$ values, *Ceriodaphnia* values being the most negative of the resting stages. Average cladoceran ephippia $\delta^{13}C$ values in the flotsam agreed well with ephippia values from Gerzensee surface sediments. In contrast, average *Plumatella* statoblast $\delta^{13}C$ values from the flotsam were 4 ‰ more negative than in the surface sediments. In the sediment core, $\delta^{13}C$ values of the two cladocerans remained low (mean -39.0 ‰ and -41.9 ‰) throughout the record. In contrast, *Plumatella* had distinctly less negative $\delta^{13}C$ values (mean -32.0 ‰). Our results indicate that in Gerzensee, *Daphnia*

45 and *Ceriodaphnia* strongly relied on a ^{13}C-depleted food source throughout the past 150
46 years, most likely methane-oxidising bacteria, whereas this food source was not a major
47 contribution to the diet of bryozoans.

48

49 ## Introduction

50

51 Chitinous remains of aquatic invertebrates are readily preserved in lake sediments and their
52 stable carbon isotope compositions, expressed as $\delta^{13}C$ values, have been used as a proxy
53 to study past changes in the availability and importance of different carbon pathways in
54 lakes (Frossard et al. 2014; Rinta et al. 2016; van Hardenbroek et al. 2010; Wooller et al.
55 2012). Two such pathways are the fixation of dissolved CO_2 by algae and the recycling of
56 carbon from methane (CH_4) by methane-oxidising bacteria (MOB). Up to now,
57 palaeolimnological studies that analysed the $\delta^{13}C$ values of chitinous invertebrate remains
58 have focused mainly on benthic invertebrates, most prominently chironomid larvae (Insecta:
59 Diptera: Chironomidae), whose chitinous head capsules are regularly found in sediments
60 (Belle et al. 2014; Heiri et al. 2012; van Hardenbroek et al. 2010). Additionally, exoskeleton
61 fragments of planktonic invertebrates such as *Bosmina* (Branchiopoda: Cladocera:
62 Bosminidae) have been analysed for their $\delta^{13}C$ values (Perga 2009, 2011). It has recently
63 been suggested that similar information may be gained from $\delta^{13}C$ analysis of resting stages
64 of planktivorous invertebrates (Schilder et al. 2015a, 2015b; van Hardenbroek et al. 2013,
65 2014; Wooller et al. 2012). Examples of these resting stages are ephippia, produced by
66 *Daphnia* (Branchiopoda: Cladocera: Daphniidae) and other planktonic cladocerans, and
67 statoblasts, produced by bryozoans that form sessile colonies on hard substrates in the
68 shallow parts of lakes (Wood and Okamura 2005). Ephippia and statoblasts are abundant in
69 lake sediments (Francis 2001; Korhola and Rautio 2001), providing a potential archive for
70 palaeoecological reconstructions.

71 In modern lake food web studies, the analysis of the $\delta^{13}C$ value of different food
72 components is a widely used technique to differentiate between carbon sources (Bunn and
73 Boon 1993). The main organic carbon sources for filter-feeding zooplankton are algae,
74 detritus, and heterotrophic bacteria (Edmondson 1957; Lampert 2011). Using characteristic

isotopic signatures of these different sources, carbon flow can be traced through a lake's food web (Fry 2006; Taipale et al. 2007). Fundamental for such studies is that the carbon isotopic composition of an organism closely reflects the isotopic signature of its diet (DeNiro and Epstein 1978; Peterson and Fry 1987). For *Daphnia* and their ephippia, Perga (2011) and Schilder et al. (2015b) showed that ephippia $\delta^{13}C$ values closely reflect the $\delta^{13}C$ values of the living *Daphnia* at the time of ephippia production. Van Hardenbroek et al. (2016) recently demonstrated that the $\delta^{13}C$ values of bryozoan statoblasts are related to the $\delta^{13}C$ values of the colonies that produced them. More work is required, however, to show that $\delta^{13}C$ values of *Daphnia* ephippia and bryozoan statoblasts reflect the diet of the parent organisms during or shortly before resting stage formation.

Understanding the extent to which lakes change their capacity to sequester or release carbon in response to global warming and eutrophication is a key issue for palaeoecologists today (Seddon et al. 2014). New insights into lake carbon cycling can be gained from analysing $\delta^{13}C$ values of aquatic invertebrate remains. Recently, it was suggested that carbon from CH_4 may be an important additional transfer pathway of carbon in lake food webs, indicated by remarkably low $\delta^{13}C$ values of aquatic invertebrates when they incorporate CH_4-derived, ^{13}C-depleted carbon (Bastviken et al. 2003; Bunn and Boon 1993; Grey et al. 2016). The $\delta^{13}C$ values of biogenic CH_4 in small European lakes range between -86 and -61 ‰ (Rinta et al. 2015), which is markedly depleted in ^{13}C relative to algae with $\delta^{13}C$ values that typically fall in the range from -35 to -25 ‰ (Jones et al. 1999; Peterson and Fry 1987). The large difference in $\delta^{13}C$ values thus allows for differentiation between photosynthetically produced organic matter and MOB as potential carbon sources for primary consumers in lakes. Significant relationships have been observed between $\delta^{13}C$ of *Daphnia* ephippia in surface sediments and diffusive CH_4 flux (Van Hardenbroek et al. 2013) and within-lake CH_4 concentrations (Schilder et al. 2015a). Cladoceran remains may thus record changes in past diffusive CH_4 flux in lakes, though they do not provide direct information on other forms of CH_4 fluxes such as ebullition or plant-mediated transport.

Palaeoecological studies using $\delta^{13}C$ values of chitinous remains have largely

overlooked the use of bryozoan statoblasts, which have been suggested as a resource for reconstructing $\delta^{13}C$ values of primary production (Turney 1999; van Hardenbroek et al. 2014, 2016). As filter feeders, Bryozoa rely on algae, particulate organic matter (POM) and associated microorganisms (e.g. bacteria) as their main food source (Kaminsky 1984). Bryozoa are mainly bound to their substrate and do not have access to food sources from deeper water layers and are therefore not, or only to a small degree, influenced by CH_4-derived carbon (van Hardenbroek et al. 2016). By combining $\delta^{13}C$ measurements of mobile filter feeders like *Daphnia* with sessile filter feeders like *Plumatella* (Phylactolaemata: Plumatellida: Plumatellidae), it is possible to separate changes in the importance of CH_4-derived carbon from changes in algal $\delta^{13}C$ values, or more generally, changes in lake productivity (van Hardenbroek et al. 2014; Rinta et al. 2016). At present, however, no information is available about seasonal changes in $\delta^{13}C$ values of cladoceran and bryozoan resting stages or about the extent to which this seasonality influences the $\delta^{13}C$ values of statoblast and ephippia remains in lake sediments. Furthermore, it is unknown whether the $\delta^{13}C$ values of deposited organism remains reflect integrated $\delta^{13}C$ values for statoblasts and ephippia that float on the lake surface, or whether the sedimentary assemblages are characterized by systematically higher or lower values. Such differences could appear as a consequence of degradation, transport processes, or production of resting stages in cryptic habitats that do not contribute to floating resting stages at the lake surface (e.g. from deeper littoral, lower epilimnetic or profundal habitats). These uncertainties currently hinder the development of $\delta^{13}C$ analysis of invertebrate resting stages as a proxy for reconstructing past variations in carbon cycling in lakes.

The main aim of this study was to assess the influence of seasonality on $\delta^{13}C$ values of zooplankton remains and evaluate their potential use as palaeoecological indicators for lacustrine carbon cycle dynamics. This study provides a first assessment of seasonal variations in $\delta^{13}C$ values of cladoceran and bryozoan resting stages in a small temperate European lake, with respect to physical, chemical and biological variables. In particular, the focus was on assessing seasonal variations in transfer pathways of carbon in the lake, and

their influence on the $\delta^{13}C$ values of organisms in the water column, as well as their chitinous resting stages (i.e. planktonic Cladocera and their ephippia, bryozoan statoblasts). In a field campaign, $\delta^{13}C$ values of *Daphnia* and their ephippia were analysed over a two-year period, and $\delta^{13}C$ values of floating resting stages of *Ceriodaphnia* (Branchiopoda: Cladocera: Daphniidae) and *Plumatella* were analysed when present during this period. In a second step, we investigated the implications of seasonal changes for interpretation of $\delta^{13}C$ values in fossil invertebrate remains. To that end, ephippia of the cladocerans *Daphnia* and *Ceriodaphnia*, and statoblasts of the bryozoan *Plumatella* were analysed in the lake surface sediment and in a short sediment core covering roughly the past 150 years. Our study was conducted on Lake Gerzensee (7°33'E, 46°50'N, 606 m a.s.l.), a small temperate lake in the foreland of the Swiss Alps, about 20 km south of Bern (Fig. 1). The lake is characterised by exceptionally high lake water CH_4 concentrations (Rinta et al. 2015; Schilder et al. 2016). Lake Gerzensee has a surface area of 0.27 km^2, a total water volume of 0.16 km^3, and a maximum depth of 10 m. The catchment area (2.6 km^2) consists of 80 % agricultural land, 5 % wooded land, and 15 % urban areas (Lotter et al. 2000). The mean annual temperature is 8.8 °C (Bern Zollikofen 1981-2010, Bundesamt für Meteorologie und Klimatologie MeteoSchweiz 2014). Today, Lake Gerzensee is eutrophic, with summer anoxia in the hypolimnion (Zeh et al. 2004).

Materials and methods

Lake Gerzensee was sampled on 15 one-to-three-day visits between October 2012 and July 2014, throughout all seasons (Electronic Supplementary Material [ESM] Table S1). In the first period, between October 2012 and September 2013, measurements were taken every other month (6 visits). Based on the first year of data, the fieldwork protocol was adjusted to gain more detailed information about deeper-water POM and chlorophyll *a* concentrations (ESM Table S1). In the second year, intervals between field visits were reduced to two weeks from September 2013 to December 2013, to cover the destratification period (4 visits),

159 and sampling continued with 2-4 week intervals after the lake ice thawed in February 2014,
160 until July 2014 (5 visits). Data for March and July 2014 were collected along with other
161 fieldwork activities, using a shortened protocol (ESM Table S1).
162
163 Sample collection
164
165 Vertical profiles of temperature and dissolved oxygen concentration ($[O_2]_{aq}$) were measured
166 for every metre in the water column at the lake centre (location C1, Fig. 1), using a multi-
167 sensor probe (WTW CellOx© 325 oxi1970i, Germany). *Daphnia* individuals were collected
168 from the oxic part of the water column at the lake centre (location C1) in multiple vertical
169 hauls, with a 40-µm mesh plankton net. Flotsam was collected from the lake surface with a
170 hand net (mesh size ~120 µm). Water samples were collected at C1 in 0.7 m and 8 m water
171 depth (top epilimnion and bottom hypolimnion, respectively), using a 5-L water sampler
172 (UWITEC, Austria). For these water samples, pH was measured (Waterproof pHTestr 20,
173 Oakton, USA), and 60 ml of water was injected with a syringe into a 118-ml glass vial
174 through a 10-mm-thick butyl rubber stopper (Apodan, Denmark) to determine the abundance
175 and $\delta^{13}C$ value of the dissolved inorganic carbon (DIC). The vials were prepared beforehand
176 with 200 µl of H_3PO_4 (85 %), closed, and repeatedly vacuumed and flushed with N_2 to
177 ensure that no CO_2 remained in the vials (Rinta et al. 2015; Schilder et al. 2015a). Samples
178 for the $\delta^{13}C$ analysis of POM were collected from the lake water at C1 (0.7 m water depth)
179 by passing the water through a 250-µm sieve before manually pushing water through a glass
180 fibre filter (Whatman GF/C 25 mm, pore size 1.2 µm) with a syringe (water volume recorded
181 in the field). A second sample of POM was obtained in a similar fashion, immediately put into
182 90 % undenatured ethanol, and covered with aluminium foil for chlorophyll *a* analysis.
183 Immediately after returning from fieldwork, POM filters designated for chlorophyll *a* analysis
184 were heated to 70 °C in 90 % undenatured ethanol for 10 min, put in an ultrasound bath for
185 5 min, and stored in a refrigerator for at least 48 hours. The samples were then filtered
186 through a membrane filter to remove suspended particles. Chlorophyll *a* measurements

were based on absorbance at characteristic wavelengths (Schwoerbel 1994). Samples were injected into glass cuvettes (1 cm light path) and absorbance at wavelengths (λ) 665 nm and 750 nm was measured with a spectrophotometer (Jenway, UK). Chlorophyll *a* content in the lake water was calculated following EAWAG (1995).

Isotope analysis

$\delta^{13}C$ analysis of DIC and POM

The concentration of CH_4 and CO_2 in the headspace of the DIC samples was measured by gas chromatography with a flame ionisation detector and methanizer (GC-FID; Shimadzu GC8, PoropackN column, see Rinta et al. 2015 for details). Lake water DIC concentrations and dissolved CH_4 concentrations ($[CH_4]_{aq}$) were back-calculated from measured headspace CO_2 and CH_4 concentrations. Dissolved CO_2 concentrations ($[CO_2]_{aq}$) (as the sum of dissolved CO_2 and H_2CO_3) were calculated following Stumm and Morgan (1996), accounting for lake water pH, temperature, and DIC concentrations.

Filters containing lake water POM were freeze-dried and a maximum amount of filter material was separated from the seston. The seston was then transferred into ultra-clean tin cups. For $\delta^{13}C$ analysis of DIC, 15-20 ml of gas from the headspace of the vial was allowed to escape into a 60-ml syringe. The gas was then injected into a pre-vacuumed 12-ml glass vial such that slight overpressure was applied. The procedure was repeated for $\delta^{13}CH_4$ measurements. Isotope samples of CO_2, CH_4, and POM were analysed at the Stable Carbon Isotope Facility of the University of California-Davis on an Elementar Vario EL Cube or Micro Cube elemental analyser interfaced to a PDZ Europa 2020 isotope ratio mass spectrometer (IRMS). Analytical uncertainties for gas sample $\delta^{13}C$ values were < 0.1 ‰ (one standard deviation) for CO_2 for two to three replicate measurements of three laboratory standards ($\delta^{13}C$ = -40.73 ‰, -10.39 ‰, and -3.59 ‰) and < 0.1 ‰ (one standard deviation) for CH_4 for replicate measurements of a laboratory standard ($\delta^{13}C$ = -36.7 ‰, n = 6). Results

are reported in conventional δ-notation relative to the international standard Vienna PeeDee Belemnite (V-PDB).

$\delta^{13}C$ analysis of zooplankton

Living *Daphnia* were kept in approximately 1 L of unfiltered lake water for 1-2 days. They were then separated from other organisms under a dissecting microscope (magnification 20x–50x), and were frozen in demineralised water. Samples were freeze-dried and weighed into ultra clean tin cups (Lüdi Swiss AG, Switzerland). Because *Daphnia* ephippia cannot be identified to species level under the microscope, the species of living *Daphnia* was not determined for this analysis. Flotsam was sieved at 100 µm and examined under a dissecting microscope. Ephippia of the planktonic cladocerans *Daphnia* and *Ceriodaphnia*, and statoblasts of the bryozoan *Plumatella* were the only invertebrate resting stages found regularly and abundantly enough for $\delta^{13}C$ analysis. These resting stages were identified according to Vandekerkhove et al. (2004) (Cladocera) and Wood and Okamura (2005) (Bryozoa), exposed to 10 % potassium hydroxide (KOH) for 2 hours (van Hardenbroek et al. 2010), rinsed 5-10 times with demineralised water, and picked into ultra-clean tin cups. Isotope samples of *Daphnia* as well as ephippia and statoblasts were also analysed at the Stable Carbon Isotope Facility of the University of California-Davis on an Elementar Vario EL Cube or Micro Cube elemental analyser interfaced to a PDZ Europa 2020 IRMS. Sample sizes were in the range of 120-250 individuals for *Daphnia* (150-500 µg), 150-200 for *Daphnia* ephippia (150-250 µg), 200-300 for *Plumatella* statoblasts (150-300 µg), and 450-600 (150-200 µg) for *Ceriodaphnia* ephippia. Analytical uncertainties for invertebrate $\delta^{13}C$ measurements were ≤ 0.6 ‰ (one standard deviation) for replicate measurements (n = 3-36) of five laboratory standards (Bovine Liver ($\delta^{13}C$ = -21.7 ‰), USGS-41 Glutamic Acid ($\delta^{13}C$ = 37.6 ‰), Nylon 5 ($\delta^{13}C$ = -27.7 ‰), Peach Leaves ($\delta^{13}C$ = -26.1 ‰), Glutamic Acid ($\delta^{13}C$ = -28.9 ‰)).

243 Sediment analysis

244

245 *Sediment coring and chronology*

246

247 In October 2012, a 62-cm-long sediment core (GER12) was recovered from the centre of
248 Lake Gerzensee (9.5 m water depth) using a gravity corer (UWITEC, Austria). Upon arrival
249 in the laboratory, core GER12 was sampled at 1-cm intervals and freeze-dried until further
250 analysis. For ^{210}Pb and ^{137}Cs dating, a total of 15 freeze-dried samples from the upper 48 cm
251 of the core were analysed using gamma spectrometry at the Department of Chemistry and
252 Biochemistry at the University of Bern, Switzerland. ^{210}Pb (46.5 keV), ^{241}Am (59.5 keV),
253 ^{226}Ra progenies (351.9 and 609.3 keV), and ^{137}Cs (661.7 keV) were measured using a
254 Broad Energy Germanium (BEGe) Canberra detector with low background and high
255 absolute full-energy peak efficiencies for close on-top geometries of > 20 % and ~ 5 % for
256 ^{210}Pb and ^{137}Cs, respectively.

257

258 *δ^{13}C analysis of sedimentary invertebrate remains*

259

260 For invertebrate δ^{13}C analysis, core GER12 was sampled every fourth centimetre. When
261 sample mass of the invertebrate remains was not sufficient for δ^{13}C analysis, the lower
262 adjacent centimetre was added to the sample. Nonetheless, some samples in the lower half
263 of the core had to be pooled with the next regular-interval sample to obtain a sufficient
264 number of remains for analysis. Hence, invertebrate δ^{13}C values represent remains from up
265 to 6 cm of sediment. For each sample, 50 % by weight of the freeze-dried material was
266 deflocculated in 10 % KOH for 2 hours and sieved at 100 µm (van Hardenbroek et al. 2010).
267 *Daphnia* ephippia, *Ceriodaphnia* ephippia, and *Plumatella* statoblasts were identified
268 according to Vandekerkhove et al. (2004) for Cladocera and Wood and Okamura (2005) for
269 Bryozoa and separated from the sediment. Remains were treated with 2M NH_4Cl solution
270 buffered with 0.35 NaOH for 20 hours to remove carbonates (Verbruggen et al. 2010), and

picked into pre-weighed silver cups (6 x 4 mm; Säntis, Switzerland). All fossil invertebrates were analysed at the Alaska Stable Isotope Facility of the University of Alaska, Fairbanks, on a Costech ESC 4010 elemental analyzer interfaced via a ThermoConflo III to a Thermo Delta V IRMS. Analytical uncertainties were < 0.1 ‰ for replicate measurements of a laboratory standard (peptone, $\delta^{13}C$ = -15.8 ‰, n = 24). Sample sizes were in the range of 20-55 remains for *Daphnia* ephippia and *Plumatella* statoblasts, and 100-200 for *Ceriodaphnia* ephippia. In addition to the sediment core, material from a sediment trap located close to the coring site at the centre of Gerzensee during 2012/13 was available from another study (C. Adolf, unpublished). The trap was placed 3 m above the lake floor and remained in the lake for 12 months. The material was processed in the same way as the sediment core, and one sample for *Ceriodaphnia* ephippia was analysed for its $\delta^{13}C$ value.

Statistical analysis

Statistical analyses on material collected alive and as floatsam were performed in R (R Development Core Team 2008). For Pearson correlation tests, normality of the variables was tested prior to analysis using the Shapiro-Wilk test. When a normal distribution of the data was rejected, Spearman's rank correlation was used to test for correlations. The average value reported for fieldwork parameters was calculated as a weighted mean of all samples collected during the campaign and each sample was weighted by the time interval for which it is most representative.

Results

Physical and chemical lake conditions

All variables showed characteristic seasonal variations throughout the two years of

298 monitoring. Measured surface water temperatures ranged between 4.6 °C in December
299 2013 and 25.8 °C in July 2013, with a mean of 12.4 °C (Fig. 2a). The bottom water (8 m
300 depth) was anoxic ($[O_2]_{aq}$ < 1 mg L^{-1}) from June to November (Fig. 2b). Chlorophyll *a* values
301 ranged from 15.2 µg chl *a* L^{-1} in autumn 2013 to 7.2 µg chl *a* L^{-1} in February 2014, but only
302 one measurement was taken during summer (July 2014). Deep-water chlorophyll *a*
303 measured at 7 m depth in May and July 2014 was comparable (July, 14.7 µg chl *a* L^{-1}) or
304 higher (May, 14.2 µg chl *a* L^{-1}) than surface water measurements (12.6 and 8.6 µg chl *a* L^{-1},
305 respectively). $[CO_2]_{aq}$ in the epilimnion ranged from peak values just after autumn mixing and
306 in spring (96.7-192.8 µM) to 9.6 µM in September (ESM Fig. S1). $[CH_4]_{aq}$ in the water
307 column reached high values (1082 µM) in the hypolimnion during summer stratification.
308 Surface water $[CH_4]_{aq}$ was highest in November during autumn turnover (49.8 µM, ESM Fig.
309 S1).
310
311 Stable carbon isotopes
312
313 With the exception of *Daphnia* ephippia, all measured variables showed seasonal variation
314 in $\delta^{13}C$ values, with the lowest values right after autumn lake mixing and throughout winter,
315 and the highest values in summer (Fig. 3a).
316 The isotopic composition of $[CO_2]_{aq}$ ($\delta^{13}C_{CO2}$) for location C1 at the lake surface was
317 most ^{13}C-depleted in February and March ($\delta^{13}C_{CO2}$ ~-21 ‰), whereas the highest $\delta^{13}C_{CO2}$
318 value of -12.1 ‰ was recorded in July 2013 (Fig. 3a). The seasonal cycle in $\delta^{13}C_{CO2}$ values
319 was less pronounced in the hypolimnion (data not shown). During summer, a vertical
320 gradient in $\delta^{13}C_{CO2}$ values developed between the epilimnion and the hypolimnion, which
321 was eliminated at lake mixing in autumn. At the lake centre, the $\delta^{13}C$ value of surface water
322 POM ($\delta^{13}C_{POM}$) was highest during summer (-28.5 ‰), and reached its minimum in March
323 2013 (-41.6 ‰, mean = -34.7 ‰; Fig. 3a). Bottom water $\delta^{13}C_{POM}$, measured at 7 m depth,
324 was in the range of surface water $\delta^{13}C_{POM}$ for measurements in April and May 2014 (ESM
325 Fig. S2d). However, during summer stratification in July 2014, bottom water $\delta^{13}C_{POM}$ values

were distinctly more negative than surface water $\delta^{13}C_{POM}$ (-38.0 ‰ and -30.0 ‰, respectively). Surface water $\delta^{13}C_{POM}$ was on average 17.0 ‰ (range 13.2 to 21.0 ‰) more negative than surface water $\delta^{13}C_{CO2}$, with the largest differences measured in March 2013 and May 2014 (Fig. 3a).

Daphnia $\delta^{13}C$ values ($\delta^{13}C_{Daph}$) in the lake centre were on average 3.4 ‰ more negative than $\delta^{13}C_{POM}$ values (Fig. 3a). The average $\delta^{13}C_{Daph}$ value was -39.4 ‰. Values ranged from -44.2 ‰ in early spring to -29.8 ‰ in summer. $\delta^{13}C_{Daph}$ values were positively correlated with chlorophyll *a* concentrations in surface water POM (Pearson correlation r = 0.86, $p < 0.05$, n = 6; ESM Fig. S2a), and $[CH_4]_{aq}$ in bottom waters (log-transformed, Pearson correlation r = 0.86, $p < 0.01$, n = 13; ESM Fig. S2b). Moreover, bottom water $[CH_4]_{aq}$ showed a negative correlation with the offset between $\delta^{13}C_{POM}$ and $\delta^{13}C_{Daph}$ ($\Delta^{13}C_{POM-Daph}$) (Spearman's rank correlation r = 0.67, $p < 0.05$, n = 13; ESM Fig. S2c).

In contrast to $\delta^{13}C_{Daph}$ values, the $\delta^{13}C$ values of floating *Daphnia* ephippia ($\delta^{13}C_{DaphFlot}$) did not show seasonal variations, but instead remained relatively constant around -39.5 ‰ (range -41.7 to -38.8 ‰, Fig. 3a and b). Interestingly, floating *Ceriodaphnia* ephippia $\delta^{13}C$ values ($\delta^{13}C_{CerioFlot}$) showed changes over time, with the lowest $\delta^{13}C$ values down to -50.1 ‰ in winter (Fig. 3b). On average, $\delta^{13}C_{CerioFlot}$ was more ^{13}C-depleted (mean = -42.9 ‰, range -50.1 to -34.3 ‰) than $\delta^{13}C_{DaphFlot}$, whereas *Plumatella* statoblast $\delta^{13}C$ ($\delta^{13}C_{PluFlot}$) was least ^{13}C-depleted (mean = -36.3 ‰, range -40.0 to -34.6 ‰). In-lake abundance of the floating remains could not be assessed by the applied sampling technique, and no structural degradation of the remains was visible under the microscope on any of the fieldwork dates. *Ceriodaphnia* ephippia from the sediment trap had a $\delta^{13}C$ value of -42.4 ‰.

Sediment core and chronology

For core GER12, activity of unsupported ^{210}Pb was transformed into an age-depth model for the upper 48 cm using the constant rate of supply (CRS) model (Appleby 2001; Appleby and Oldfield 1978) (Fig. 4). Activity measurements of ^{137}Cs showed a distinct peak at 28.5 cm

and a second smaller peak at 20.5 cm. Many European lakes show two maxima in ^{137}Cs activity, which are associated with aboveground nuclear bomb tests in AD 1963 and the Chernobyl reactor accident in AD 1986 (Appleby 2001). For Swiss lakes, the latter peak is usually greater than the first (Albrecht et al. 1998; Lotter et al. 1997). This was not the case in Gerzensee sediments where only one measurement point defined the anticipated Chernobyl peak. The Chernobyl peak may thus not be fully revealed in the record because of the relatively low sampling resolution, i.e. every 4 cm. Therefore the ^{210}Pb model was not modified to fit the ^{137}Cs profile. The lower peak in ^{137}Cs activity, expected to coincide with the nuclear bomb peak in AD 1963, was confirmed by ^{241}Am and agrees well with the ^{210}Pb chronology (Fig. 4).

The abundance of invertebrate remains in the sediments of Gerzensee allowed for the analysis of eight samples of *Daphnia* ephippia covering the upper 27 cm, nine samples of *Plumatella* statoblasts (0-43 cm), and 15 samples of *Ceriodaphnia* ephippia (0-62 cm). The $\delta^{13}C$ values of all three invertebrate genera showed only small variations throughout the record (*Daphnia* ephippia -39.8 to -37.9 ‰, *Ceriodaphnia* ephippia -43.3 to -39.2 ‰, and *Plumatella* statoblasts -33.3 to -29.7 ‰; Fig. 5). $\delta^{13}C$ values of *Plumatella* statoblasts were on average distinctly less negative (-32.0 ‰) than the remains of the two mobile filter feeders *Daphnia* and *Ceriodaphnia* (-39.0 ‰ and -41.9 ‰, respectively). The most negative $\delta^{13}C$ values were measured for *Ceriodaphnia* ephippia with the minimum of -43.3 ‰ at 41-42 cm depth (AD ~1920). Remains of *Daphnia* and *Ceriodaphnia* showed a very similar pattern, with one distinct peak of less negative $\delta^{13}C$ values at about 18 cm depth (AD ~1980). In contrast, $\delta^{13}C$ values of *Plumatella* statoblasts remained constant during this period, but show a 3 ‰ increase around 30 cm (AD ~1955). Besides differences in $\delta^{13}C$ values, *Plumatella* statoblasts had a lower C:N ratio (mean = 4.3) compared to values for *Daphnia* and *Ceriodaphnia* ephippia (6.3 and 6.5, respectively).

Discussion

381

382 Seasonal variation in *Daphnia* $\delta^{13}C$ values

383

384 *Daphnia* $\delta^{13}C$ values were consistently below -38.5 ‰ and reached minimum values, less
385 than -44 ‰, in the period from autumn to spring. This is well below commonly reported
386 values for algae, which usually lie above -35 ‰ (France 1995; Peterson and Fry 1987;
387 Vuorio et al. 2006). Nonetheless, $\delta^{13}C_{Daph}$ values closely track the seasonal cycle of surface
388 water $\delta^{13}C_{POM}$ values (Fig. 3a), but with an average difference of 3.4 ‰ between $\delta^{13}C_{POM}$
389 and $\delta^{13}C_{Daph}$. Hence, $\Delta^{13}C_{POM\text{-}Daph}$ was slightly higher than values reported by del Giorgio and
390 France (1996) for zooplankton in Canadian lakes and other published values discussed by
391 these authors (mean difference 2.6 ‰). A positive correlation between $\delta^{13}C_{Daph}$ values and
392 surface water chlorophyll *a* concentrations, which can serve as a proxy for algal
393 concentrations (Sartory and Grobbelaar 1984), was observed between autumn and spring
394 (ESM Fig. S2a). Hence, low $\delta^{13}C_{Daph}$ values coincide with relatively low concentrations of
395 algae in surface water POM. During times when algae are less abundant, other food sources
396 may contribute to the carbon uptake of *Daphnia* (Taipale et al. 2008). Several studies have
397 shown that *Daphnia* and other invertebrates can incorporate CH_4-derived carbon to
398 supplement their diet (Bastviken et al. 2003; Devlin et al. 2015; Kankaala et al. 2006). In a
399 study of a small Finnish lake, Taipale et al. (2008) found that MOB contributed to *Daphnia*'s
400 diet throughout the year, comprising up to 50 % of the diet in autumn. Considering the high
401 CH_4 concentrations in Gerzensee, uptake of ^{13}C-depleted carbon by feeding on MOB seems
402 the likely explanation for the exceptionally low $\delta^{13}C_{Daph}$ values measured in Gerzensee.

403

404 The relationship between *Daphnia* $\delta^{13}C$ values and $[CH_4]_{aq}$

405

406 Field studies that compared the carbon isotopic composition of invertebrate remains in
407 surface sediment samples with in-lake CH_4 abundance suggest that a relationship exists
408 between the $\delta^{13}C$ values of some invertebrate groups and lake water CH_4 concentrations

409 (Schilder et al. 2015a; van Hardenbroek et al. 2013). Within our two-year measurement
410 period at Gerzensee, $\delta^{13}C_{Daph}$ was positively correlated with $[CH_4]_{aq}$ in the bottom waters, i.e.
411 we recorded $\delta^{13}C_{Daph}$ values more similar to $\delta^{13}C_{POM}$ during times of high bottom water
412 $[CH_4]_{aq}$ (ESM Fig. S2b). This is also indicated by the negative correlation between $[CH_4]_{aq}$ in
413 the bottom waters and $\Delta^{13}C_{POM\text{-}Daph}$ (ESM Fig. S2c). Hence, during lake stratification, when
414 $[CH_4]_{aq}$ is increasing in the hypolimnion, *Daphnia* utilise carbon whose isotopic signature is
415 more similar to $\delta^{13}C_{POM}$ than at times when the water column is mixed. This suggests that
416 during lake stratification, algae are the main food source of *Daphnia*, whereas *Daphnia* may
417 rely more heavily on other food sources when the lake is mixed. In a multi-lake study,
418 Schilder et al. (2015a) found a negative correlation between late summer $[CH_4]_{aq}$ in both
419 bottom and surface waters, and $\delta^{13}C$ values of *Daphnia* ephippia isolated from surface
420 sediment samples, indicating that *Daphnia* are more likely to incorporate CH_4-derived carbon
421 in lakes with high CH_4 abundance. With regard to this relationship, our results imply that in
422 lakes with high CH_4 accumulation during summer stratification, more ^{13}C-depleted carbon
423 becomes accessible to the food web upon mixing and oxygenation of CH_4 in the water
424 column, and this signal is seen in the floating ephippia. Other processes, e.g. increased
425 transport of allochthonous organic carbon and higher associated CH_4 production may also
426 contribute to the importance of CH_4-derived carbon for *Daphnia* in the autumn months.
427
428 Floating cladoceran and bryozoan remains
429
430 In a field study, Perga (2011) showed that there is no significant carbon isotope fractionation
431 between *Daphnia* and their ephippia. This was confirmed in a laboratory experiment by
432 Schilder et al. (2015b), which showed that *Daphnia* ephippia $\delta^{13}C$ values closely resemble
433 those of *Daphnia* during ephippia production under different environmental conditions (-0.2
434 ±0.4 ‰ for 12°C, 1.3 ±0.3 ‰ for 20°C). In Gerzensee, $\delta^{13}C_{DaphFlot}$ remained very similar
435 across the annual cycle (Figs. 3a, b). If no fractionation is assumed during ephippia
436 production, this suggests that the ephippia were produced batch-wise and then floated on

437 the lake for several months. In the data set presented here, periods when $\delta^{13}C_{DaphFlot}$
438 coincided with $\delta^{13}C_{Daph}$ include December 2012, June 2013, November 2013, and April 2014.
439 This is in line with the observation that ephippia production commonly occurs in early
440 summer and late autumn (Cáceres 1998), when environmental conditions deteriorate, e.g.
441 there is temperature decline, crowding, oxygen depletion, or limited food availability (Korhola
442 and Rautio 2001). Hence, in Gerzensee, changes in $\delta^{13}C_{DaphFlot}$ reflect $\delta^{13}C_{Daph}$ during
443 ephippia production, i.e. in late autumn and/or early spring, but $\delta^{13}C_{DaphFlot}$ does not reflect
444 seasonal changes in the diet of *Daphnia*. This observation differs from the findings of
445 Schilder (2014) at Dutch Lake De Waay, where *Daphnia* ephippia followed the seasonal
446 variation of *Daphnia* $\delta^{13}C$, indicating several production periods per year. The reason for the
447 different timing of ephippia production is unclear, although it could be explained by
448 differences in species composition between the two lakes. Regardless of the cause, our
449 results indicate that different production intervals, and possibly switching between different
450 production intervals over time, potentially influences *Daphnia* ephippia $\delta^{13}C$ values in
451 sediment records.

452 Floating *Ceriodaphnia* ephippia show larger variability in $\delta^{13}C$ values than *Daphnia*
453 ephippia (Fig. 3b). To our knowledge, no prior study has assessed the fractionation between
454 maternal *Ceriodaphnia* and the chitinous structure of their resting eggs. Thus, following the
455 simplest assumption of no (or constant) fractionation, as is observed for *Daphnia*, our results
456 suggest that *Ceriodaphnia* ephippia are produced at several times or continuously
457 throughout the year. No living *Ceriodaphnia* were analysed in this study, but the broad range
458 of $\delta^{13}C_{CerioFlot}$ values (-34.3 to -50.1 ‰), which is comparable in span to the seasonal cycle of
459 living *Daphnia* (-29.8 to -44.2 ‰), may be an indication that the changes in $\delta^{13}C_{CerioFlot}$
460 represent (a part of) the seasonal cycle of *Ceriodaphnia* $\delta^{13}C$.

461 Floating *Plumatella* statoblast $\delta^{13}C$ values also show more pronounced seasonal
462 changes than $\delta^{13}C_{DaphFlot}$, but the variability is not as large as for $\delta^{13}C_{CerioFlot}$ (Fig. 3b). Since
463 no living *Plumatella* zooids were analysed, no pattern of statoblast production can be
464 determined. However, a recent study by van Hardenbroek et al. (2016) suggests that the

465 $\delta^{13}C$ values of *Plumatella* statoblasts collected from colonies are significantly correlated with
466 *Plumatella* zooid $\delta^{13}C$. Okamura and Hatton-Ellis (1995) found that production of statoblasts
467 may start in mid-summer, but highest production occurs in late summer and early autumn.
468 Overwintering statoblasts are released when colonies collapse in late autumn. Following
469 these findings, $\delta^{13}C$ values of floating *Plumatella* statoblasts may reflect zooid $\delta^{13}C$ values
470 during statoblast production from late summer to late autumn.
471
472 Linking $\delta^{13}C$ values of floating resting stages to those of fossil invertebrate remains
473
474 The average $\delta^{13}C$ value of floating *Daphnia* ephippia (-39.5 ‰ ± 0.9 [one standard
475 deviation]) is in excellent agreement with the $\delta^{13}C$ value of *Daphnia* ephippia in the
476 uppermost 1-3 cm of the sediment at the centre of the lake (-39.8 ‰; Fig. 5), and with the
477 average $\delta^{13}C$ value of living *Daphnia* collected during the two-year fieldwork period (-39.4 ‰
478 ± 4.6). This confirms that $\delta^{13}C$ values of *Daphnia* ephippia in the sediment of Gerzensee
479 reflect the $\delta^{13}C$ signal of floating *Daphnia* ephippia during the year. Seasonal changes in
480 $\delta^{13}C_{Daph}$, however, are not recorded by floating or deposited *Daphnia* ephippia $\delta^{13}C$ values in
481 the lake (see previous sections). This has implications for the interpretation of the sediment
482 core record of *Daphnia* ephippia $\delta^{13}C$ values. In Gerzensee, nearly constant $\delta^{13}C_{DaphFlot}$
483 values throughout the year indicate batch-wise production of ephippia in autumn and/or early
484 spring. Changes in the $\delta^{13}C$ value of sedimentary *Daphnia* ephippia may thus record
485 changes in the importance of MOB in the diet of *Daphnia* (only) during spring and/or autumn.
486 The latter period is indirectly coupled with the accumulation of $[CH_4]_{aq}$ during summer
487 stratification, which is the reason for high CH_4 abundance in the water column during autumn
488 lake mixing. Hence $\delta^{13}C$ values of sedimentary *Daphnia* ephippia may indicate the
489 accumulation of $[CH_4]_{aq}$ during summer stratification in small European lakes like Lake
490 Gerzensee, as suggested by Schilder et al. (2015a).
491 The *Ceriodaphnia* ephippia $\delta^{13}C$ value recorded in the sediment trap during 2012/13 (-
492 42.5 ‰) is in excellent agreement with the surface sediment measurement presented here (-

493 42.9 ‰, 1-3 cm; Fig. 5). The average flotsam $\delta^{13}C$ value (-42.9 ‰ ± 4.8) also agrees very
494 well with the surface sediment measurement, although no full annual cycle is covered by the
495 flotsam measurements (October to May only, Fig. 3b). Our study is the first to assess and
496 confirm that the $\delta^{13}C$ values of *Ceriodaphnia* ephippia in the sediment can provide
497 information about the $\delta^{13}C$ value of floating ephippia at the time of deposition.
498 The *Plumatella* statoblast $\delta^{13}C$ value in the surface sediment (-32.7 ‰, pooled 1-3 and
499 5-7 cm, this study) was distinctly less negative than the average $\delta^{13}C$ value of floating
500 statoblasts (-36.3 ‰ ± 1.7; Fig. 5). It should, however, be noted here that summer
501 measurements of $\delta^{13}C_{PluFlot}$ are underrepresented in our dataset, with only one measurement
502 in July 2013 (Fig. 3b). It therefore remains uncertain whether a better representation of the
503 summer period may resolve this mismatch between average flotsam values and the surface
504 sediment measurement.
505
506 Interpreting fossil invertebrate $\delta^{13}C$ values
507
508 *Daphnia* ephippia $\delta^{13}C$ values in our sediment record (-39.8 to -37.9 ‰) were continuously
509 below -35 ‰, whereas algae are commonly reported to have $\delta^{13}C$ values above -35 ‰
510 (France 1995; Peterson and Fry 1987; Vuorio et al. 2006). Our values are comparable to
511 sedimentary *Daphnia* ephippia $\delta^{13}C$ values found in Lake De Waay, the Netherlands
512 (Schilder 2014), but are distinctly more negative than cladoceran $\delta^{13}C$ values reported from
513 sediment records of Lake Strandsjön, Sweden (van Hardenbroek et al. 2014) and Lake
514 Annecy, France (Frossard et al. 2014). In sediment records covering longer time periods,
515 van Hardenbroek et al. (2013) and Wooller et al. (2012) found $\delta^{13}C$ values over the range of
516 all of the studies mentioned above. In a recent study in Lake Mekkojärvi, Finland, Rinta et al.
517 (2016) showed that *Daphnia* ephippia $\delta^{13}C$ values changed abruptly from values below -
518 45 ‰ to values greater than -40 ‰, shifts that these authors interpreted as representing
519 changes in the availability of CH_4 in this lake. As discussed earlier, $\delta^{13}C$ values in *Daphnia*
520 ephippia have been shown to correlate negatively with CH_4 abundance in the hypolimnia of

small European lakes (Schilder et al. 2015a). Therefore, the observed *Daphnia* ephippia $\delta^{13}C$ values below -35 ‰ suggest that $[CH_4]_{aq}$ remained high at Gerzensee throughout the record. Only the least negative $\delta^{13}C$ values, around 20 cm (AD ~1970-1980), could indicate a short period with a reduced influence of ^{13}C-depleted carbon in the diet of *Daphnia*, but $\delta^{13}C$ values remained clearly more negative than commonly reported algal $\delta^{13}C$ values. Lower *Daphnia* $\delta^{13}C$ values observed for the sediments of eutrophic and stratified lakes Gerzensee and De Waay can potentially be explained by a greater importance of CH_4-derived carbon compared to that in less nutrient-rich Lake Annecy and non-stratified Lake Strandsjön, where algae are the main carbon source of *Daphnia.* Mekkojärvi is a very small, stratified, humic lake with low oxygen concentrations within 1 m of the lake surface (Rinta et al. 2015), which may explain the high relevance of CH_4-derived carbon in its planktonic food web.

Ceriodaphnia ephippia were not analysed in any of the above-mentioned studies. *Ceriodaphnia* ephippia, however, had even more negative $\delta^{13}C$ values than *Daphnia* in the Gerzensee sediment (range -43.3 to -39.4 ‰; Fig. 5). This indicates a similar, but potentially more dominant source of ^{13}C-depleted carbon for *Ceriodaphnia* ephippia. The systematic ^{13}C-depletion of *Ceriodaphnia* ephippia relative to *Daphnia* ephippia may be caused by a difference in the average particle size that these taxa filter from the water. *Ceriodaphnia* feed on smaller particles than *Daphnia* (Geller and Müller 1981), potentially containing a higher proportion of MOB and other bacteria. The relative ^{13}C-depletion of *Ceriodaphnia* could also be caused by differences in body composition, e.g. lipid content, between taxa, as has been suggested by Matthews and Mazumder (2005) for other zooplankton species. However, C:N ratios, which may be an indicator of lipid content (Matthews and Mazumder 2005), do not show large differences between *Daphnia* and *Ceriodaphnia* ephippia (mean = 6.3 and 6.4 for the uppermost 27 cm of the sediment record, respectively). Therefore, low $\delta^{13}C$ values throughout the record suggest that, similar to *Daphnia*, *Ceriodaphnia* rather consistently incorporated a ^{13}C-depleted carbon source over the past 150+ years, at least during the season(s) of ephippia production.

549 *Plumatella* statoblast $\delta^{13}C$ values in our sediment record were distinctly less negative
550 compared to *Daphnia* and *Ceriodaphnia* ephippia (-33.3 to -29.7 ‰; Fig. 5). This suggests
551 that *Plumatella* zooids accessed carbon sources that were less ^{13}C-depleted compared to
552 those on which the two cladoceran taxa relied. Similar $\delta^{13}C$ values were reported by van
553 Hardenbroek et al. (2014), for *Plumatella* statoblasts in shallow Lake Strandsjön in Sweden
554 (-33.1 to -28.0 ‰). Our $\delta^{13}C$ values for *Plumatella* statoblasts are well in the range of
555 commonly reported $\delta^{13}C$ values for algae. Hence, it appears that $\delta^{13}C$ values of sedimentary
556 *Plumatella* statoblasts can potentially provide information about the temporal evolution of
557 algal $\delta^{13}C$, and thus help track past changes in the lake's carbon cycle. However, a single
558 statoblast flotsam sample from our study, collected during autumn mixing in November 2013
559 (Fig. 3b), yielded a $\delta^{13}C$ value of -40.0 ‰, well below the values expected for algae in Lake
560 Gerzensee. Similarly, individual bryozoan samples with very negative $\delta^{13}C$ values have
561 been reported in previous studies (e.g. Rinta et al. 2016; van Hardenbroek et al. 2016). This
562 suggests that these organisms may also ingest ^{13}C-depleted carbon sources such as MOB
563 under circumstances when they are abundant in their habitats.
564 In the sediment core from Gerzensee, *Daphnia* and *Ceriodaphnia* ephippia show very
565 similar variations in their $\delta^{13}C$ values in the part of the core where both groups are present
566 (0-27 cm; Fig. 5). This suggests that *Daphnia* and *Ceriodaphnia* were subject to similar
567 changes in their diet over time. In contrast, *Plumatella* statoblasts show a peak in $\delta^{13}C$
568 values that does not coincide with the peak in the ephippia of *Daphnia* and *Ceriodaphnia*.
569 The two groups also differ in the range of their $\delta^{13}C$ values, which points towards different
570 food sources for *Plumatella* and the cladoceran taxa. Access to different carbon sources
571 may occur because of differences in both habitat and particle-size preference. *Plumatella*
572 are sessile organisms in shallow areas of lakes, whereas free-living invertebrates like
573 *Daphnia* and *Ceriodaphnia* can reach deep-water food sources. Zooplankton in deeper
574 water layers may feed directly on MOB, or on other organisms that feed on MOB (e.g.
575 ciliates), which can lead to distinctly negative zooplankton $\delta^{13}C$ values (Jones and Grey
576 2011). In addition, Kaminski (1984) estimated that *Plumatella* can ingest particles between 5

577 and 17 µm in diameter, whereas *Daphnia* are able to filter particles between 0.5 and 30 µm
578 in diameter (Geller and Müller 1981). Hence, bacteria (< 2 µm) that are not attached to
579 larger particles may be too small to be caught in the lophophores of *Plumatella*, but may be
580 accessible as a food source for *Daphnia*. An alternative explanation for the observed
581 differences between cladoceran and bryozoan remains may be that both groups rely on the
582 same carbon sources (e.g. algae, detritus, heterotrophic bacteria, and possibly MOB), but
583 their relative importance in the diet is different. Whether one, both or more reasons are
584 causing the observed differences in $\delta^{13}C$ values cannot be conclusively answered by this
585 study.
586
587 **Conclusions**
588
589 In Gerzensee, *Daphnia* $\delta^{13}C$ values closely follow the seasonal cycle of POM $\delta^{13}C$ values,
590 with the most negative values of *Daphnia*, down to -44.2 ‰, measured in early spring.
591 Interestingly, *Daphnia* ephippia $\delta^{13}C$ values did not show any pronounced seasonal
592 variations. Seasonal changes in *Daphnia* $\delta^{13}C$ values, and hence potential seasonal
593 changes in the diet of *Daphnia*, are therefore not recorded in the $\delta^{13}C$ values of *Daphnia*
594 ephippia in the sediments of Gerzensee. Very low $\delta^{13}C$ values of *Ceriodaphnia* ephippia in
595 the flotsam, especially in winter (-50.1 ‰), confirm that there is a contribution of CH_4-derived
596 carbon to the diet of the crustacean zooplankton in Gerzensee. *Plumatella* statoblasts are
597 less depleted in ^{13}C than the two cladoceran taxa, indicating little or no influence of CH_4-
598 derived carbon in the diet of the bryozoan, at least during the time(s) when statoblasts are
599 produced.
600 The average *Ceriodaphnia* and *Daphnia* ephippia $\delta^{13}C$ values in the flotsam agree
601 very well with the values in surface sediments, and for *Ceriodaphnia*, with measurements
602 from a sediment trap. This shows that in Gerzensee the $\delta^{13}C$ values of ephippia in the
603 surface sediment, and potentially in general at a certain sediment depth, reflect an

integrated value for ephippia floating on the lake during a particular time span, possibly over one to two years. In contrast, average flotsam *Plumatella* statoblast $\delta^{13}C$ values were about 4 ‰ more negative than the surface sediment measurement. The reason for this mismatch may be an under-representation of the summer period in our data set. Nonetheless, this issue deserves further investigation in Gerzensee and other lakes.

Low $\delta^{13}C$ values of the two cladocerans throughout the 62-cm-long sediment core, comparable to those measured in the flotsam, indicate that in Gerzensee, *Daphnia* and *Ceriodaphnia* relied on a ^{13}C-depleted carbon source to supplement their diet throughout the past 150 years. *Daphnia* and *Ceriodaphnia* ephippia $\delta^{13}C$ values showed similar variations, with the highest values for the two taxa around the same depth. This suggests that *Daphnia* and *Ceriodaphnia* have been subject to similar changes in their diet over time. In contrast, *Plumatella* statoblast $\delta^{13}C$ values in the sediment record were again distinctly less negative, and showed a 3 ‰ increase at a different depth than the $\delta^{13}C$ values of the two cladocerans. This difference might be explained, in part, by the feeding behaviour and different habitats of the organism groups, which influences the extent to which they can incorporate CH_4-derived carbon. Our study confirms the findings of earlier studies, which indicated that taxon-specific $\delta^{13}C$ values measured on aquatic invertebrate remains can provide insights into long-term changes in the relative importance of different carbon sources. This technique can be applied to better understand the impacts of, for example, land-use change, eutrophication, and climate change on the carbon cycling in lakes. Additional studies similar to the one presented here will be needed to further constrain the effects of seasonality on the $\delta^{13}C$ values of fossil statoblast and ephippia samples, and to assess the extent to which seasonal patterns observed in Gerzensee are representative of those in other lake ecosystems.

Acknowledgements

We thank Clemens Stampfli, Studienzentrum Gerzensee and the Stiftung der

631 Schweizerischen Nationalbank for granting access to Gerzensee, Daniel Steiner for
632 assistance with chlorophyll *a* measurements and Carole Adolf for providing the sediment
633 trap material. This research was supported by the European Research Council under the
634 European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement
635 n. 239858 (RECONMET).

636

637 **References**

638

639 Albrecht A, Reiser R, Lück A, Stoll J-MA, Giger W (1998) Radiocesium dating of sediments
640 from lakes and reservoirs of different hydrological regimes. Environ Sci Technol 32:1882-
641 1887

642 Appleby PG (2001) Chronostratigraphic techniques in recent sediments. In: Last WM, Smol
643 JP (eds) Tracking environmental change using lake sediments. Volume 1: Basin analysis,
644 coring, and chronological techniques. Kluwer Academic Publishers, Dordrecht, the
645 Netherlands, pp 171-203

646 Appleby PG, Oldfield F (1978) The calculation of lead-210 dates assuming a constant rate of
647 supply of unsupported ^{210}Pb to the sediment. Catena 5:1-8

648 Bastviken D, Ejlertsson J, Sundh I, Tranvik LJ (2003) Methane as a source of carbon and
649 energy for lake pelagic food webs. Ecology 84:969-981

650 Belle S, Parent C, Frossard V, Verneaux V, Millet L, Chronopoulou P-M, Sabatier P, Magny
651 M (2014) Temporal changes in the contribution of methane-oxidizing bacteria to the biomass
652 of chironomid larvae determined using stable carbon isotopes and ancient DNA. J
653 Paleolimnol 52:215-228

654 Bundesamt für Meteorologie und Klimatologie MeteoSchweiz (2014) Homogene
655 Monatsdaten. Available at:

http://www.meteoschweiz.admin.ch/home/klima/vergangenheit/homogene-monatsdaten.html (2015)

Bunn SE, Boon PI (1993) What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. Oecologia 96:85-94

Cáceres CE (1998) Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. Ecology 79:1699-1710

Del Giorgio PA, France RL (1996) Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}C$. Limnol Oceanogr 41:359-365

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495-506

Devlin SP, Saarenheimo J, Syväranta J, Jones RI (2015) Top consumer abundance influences lake methane efflux. Nat Commun 6:8787

EAWAG (1995) Protokoll zur Bestimmung von Chlorophyll *a* mit Photometrie. Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Dübendorf, Switzerland

Edmondson WT (1957) Trophic relations of the zooplankton. Trans Am Microsc Soc 76:225-245

France RL (1995) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol Oceanogr 40:1310-1313

Francis DR (2001) Bryozoan statoblasts. In Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments, Volume 4: Zoological Indicators. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 105-123

Frossard V, Verneaux V, Millet L, Jenny J-P, Arnaud F, Magny M, Perga M-E (2014) Reconstructing long-term changes (150 years) in the carbon cycle of a clear-water lake based on the stable carbon isotope composition ($\delta^{13}C$) of chironomid and cladoceran subfossil remains. Freshw Biol 59:789-802

681 Fry B (2006) Stable Isotope Ecology, Springer, New York, USA

682 Geller W, Müller H (1981) The filtration apparatus of Cladocera: Filter mesh-sizes and their
683 implications on food selectivity. Oecologia 49:316-321

684 Grey J (2016) The incredible lightness of being methane-fuelled: stable isotopes reveal
685 alternative energy pathways in aquatic ecosystems and beyond. Front Ecol Evol 4(8):1-14

686 Heiri O, Schilder J, van Hardenbroek M (2012) Stable isotopic analysis of fossil chironomids
687 as an approach to environmental reconstruction: state of development and future challenges.
688 Fauna Nor 31:7-18

689 Jones RI, Grey J (2011) Biogenic methane in freshwater food webs. Freshw Biol 56:213-229

690 Jones RI, Grey J, Sleep D, Arvola L (1999) Stable isotope analysis of zooplankton carbon
691 nutrition in humic lakes. OIKOS 86:97-104

692 Kaminski M (1984) Food composition of three bryozoan species (Bryozoa, Phylactolaemata)
693 in a mesotrophic lake. Pol Arch Hydrobiol 31:45-53

694 Kankaala P, Taipale S, Grey J, Sonninen E, Arvola L, Jones RI (2006) Experimental $\delta^{13}C$
695 evidence for a contribution of methane to pelagic food webs in lakes. Limnol Oceanogr
696 51:2821-2827

697 Korhola A, Rautio M (2001) Cladocera and other branchiopod crustaceans. In: Smol JP,
698 Birks HJB, Last WM (eds) Tracking environmental change using lake sediments, Volume 4:
699 Zoological Indicators. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 5-41

700 Lampert W (2011) *Daphnia:* development of a model organism in ecology and evolution.
701 International Ecology Institute, Oldendorf/Luhe, Germany

702 Lotter AF, Sturm M, Teranes JL, Wehrli B (1997) Varve formation since 1885 and high-
703 resolution varve analyses in hypertrophic Baldeggersee (Switzerland). Aquat Sci 59:304-325

704 Lotter AF, Birks HJB, Eicher U, Hofmann W, Schwander J, Wick L (2000) Younger Dryas
705 and Allerød summer temperatures at Gerzensee (Switzerland) inferred from fossil pollen and
706 cladoceran assemblages. Palaeogeogr Palaeoclimatol Palaeoecol 159:349-361

707 Matthews B, Mazumder A (2005) Temporal variation in body composition (C:N) helps
708 explain seasonal patterns of zooplankton $\delta^{13}C$. Freshw Biol 50:502-515

709 Okamura B, Hatton-Ellis T (1995) Population biology of bryozoans: correlates of sessile,
710 colonial life histories in freshwater habitats. Experientia 51:510-525

711 Perga M-E (2009) Potential of $\delta^{13}C$ and $\delta^{15}N$ of cladoceran subfossil exoskeletons for paleo-
712 ecological studies. J Paleolimnol 44:387-395

713 Perga M-E (2011) Taphonomic and early diagenetic effects on the C and N stable isotope
714 composition of cladoceran remains: implications for paleoecological studies. J Paleolimnol
715 46:203-213

716 Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst
717 18:293-320

718 R Development Core Team (2008) R: A language and environment for statistical computing.
719 R Foundation for Statistical Computing. Available at: http://www.r-project.org

720 Rinta P, Bastviken D, van Hardenbroek M, Kankaala P, Leuenberger M, Schilder J, Stötter T,
721 Heiri O (2015) An inter-regional assessment of concentrations and $\delta^{13}C$ values of methane
722 and dissolved inorganic carbon in small European lakes. Aquat Sci 77:667-680

723 Rinta P, van Hardenbroek M, Jones RI, Kankaala P, Rey F, Szidat S, Wooller MJ, Heiri O
724 (2016) Land use affects carbon sources to the pelagic food web in a small boreal lake. PLoS
725 ONE 11(8):e0159900

726 Sartory DP, Grobbelaar JU (1984) Extraction of chlorophyll *a* from freshwater phytoplankton
727 for spectrophotometric analysis. Hydrobiologia 14:177-187

728 Schilder J (2014) Methanogenic carbon in *Daphnia* tissue as an indicator of lacustrine
729 methane budgets. Observations from the field, experiments and fossil records. PhD-Thesis,
730 University of Bern

731 Schilder J, Bastviken D, van Hardenbroek M, Heiri O (2016) Spatiotemporal patterns in
732 methane flux and gas transfer velocity at low wind speeds: Implications for upscaling studies
733 on small lakes. J Geophys Res Biogeosci 121:1-12

734 Schilder J, Bastviken D, van Hardenbroek M, Leuenberger M, Rinta P, Stötter T, Heiri O
735 (2015a) The $\delta^{13}C$ values of *Daphnia* ephippia reflect in-lake methane availability. Limnol
736 Oceanogr 60:1064-1075

737 Schilder J, Tellenbach C, Möst M, Spaak P, van Hardenbroek M, Wooller M, Heiri O (2015b)
738 The stable isotopic composition of *Daphnia* ephippia reflects changes in $\delta^{13}C$ and $\delta^{18}O$
739 values of food and water. Biogeosciences 12:3819-3830

740 Schwoerbel J (1994) Methoden der Hydrobiologie, Süßwasserbiologie. Gustav Fischer
741 Verlag, Stuttgart, Germany (UTB 979).

742 Seddon AW, Mackay AW, Baker AG, Birks HJB, Breman E, Buck CE, Ellis EC, Froyd CA,
743 Gill JL, Gillson L (2014) Looking forward through the past: Identification of 50 priority
744 research questions in palaeoecology. J Ecol 101(1):256-267

745 Stumm W, Morgan JJ (1996) Aquatic chemistry: chemical equilibria and rates in natural
746 waters. Wiley, New York, USA

747 Taipale S, Kankaala P, Jones RI (2007) Contributions of different organic carbon sources to
748 *Daphnia* in the pelagic foodweb of a small polyhumic lake: Results from mesocosm $DI^{13}C$-
749 additions. Ecosystems 10:757-772

750 Taipale S, Kankaala P, Tiirola M, Jones RI (2008) Whole-lake dissolved inorganic ^{13}C
751 additions reveal seasonal shifts in zooplankton diet. Ecology 89:463-474

752 Turney CSM (1999) Lacustrine bulk organic $\delta^{13}C$ in the British Isles during the last glacial –
753 Holocene transition (14-9 ka ^{14}C BP). Arct, Antarc, Alp Res 31:71-81

754 Vandekerkhove J, Declerck S, Vanhove M, Brendonck L, Jeppesen E, Porcuna Conde JM,
755 De Meester L (2004) Use of ephippial morphology to assess richness of anomopods:
756 potentials and pitfalls. J Limnol 63 (Suppl. 1):75-84

757 Van Hardenbroek M, Leuenberger M, Hartikainen H, Okamura B, Heiri O (2016) Bryozoan
758 stable carbon and hydrogen isotopes: relationships between the isotopic composition of
759 zooids, statoblasts and lake water. Hydrobiologia 765:209-223

760 Van Hardenbroek M, Lotter AF, Bastviken D, Andersen TJ, Heiri O (2014) Taxon-specific
761 $\delta^{13}C$ analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden. J
762 Paleolimnol 52:95-105

763 Van Hardenbroek M, Heiri O, Grey J, Bodelier PLE, Verbruggen F, Lotter AF (2010) Fossil
764 chironomid $\delta^{13}C$ as a proxy for past methanogenic contribution to benthic food webs in
765 lakes? J Paleolimnol, 43:235-245

766 Van Hardenbroek M, Heiri O, Parmentier FJW, Bastviken D, Ilyashuk BP, Wiklund JA, Hall
767 RI, Lotter AF (2013) Evidence for past variations in methane availability in a Siberian
768 thermokarst lake based on $\delta^{13}C$ of chitinous invertebrate remains. Quat Sci Rev 66:74-84

769 Verbruggen F, Heiri O, Reichart G-J, Lotter AF (2010) Chironomid $\delta^{18}O$ as a proxy for past
770 lake water $\delta^{18}O$: A lateglacial record from Rotsee (Switzerland). Quat Sci Rev 29:2271-2279

771 Vuorio K, Meili M, Sarvala J (2006) Taxon-specific variation in the stable isotopic signatures
772 (d13C and d15N) of lake phytoplankton. Freshw Biol 51:807-822

773 Wood TS, Okamura B (2005) A new key to the freshwater bryozoans of Britain, Ireland and
774 Continental Europe, with notes on their ecology. Freshwater Biological Association, London,
775 UK

776 Wooller MJ, Pohlman JW, Gaglioti BV, Langdon P, Jones M, Walter Anthony KM, Becker
777 KW, Hinrichs K-U, Elvert M (2012) Reconstruction of past methane availability in an Arctic
778 Alaska wetland indicates climate influenced methane release during the past ~12,000 years.
779 J Paleolimnol 48:27-42

780 Zeh M, Scheidegger D, Hoehn E, Zbären D, Maurer V (2004) Kleinseen 2003. Amt für
781 Gewässerschutz und Abfallwirtschaft des Kantons Bern, Gewässer- und Bodenschutzlabor
782 (GBL) (eds), Bern, Switzerland

783

784 **Figure Captions**

785

786 **Fig. 1** Map of Switzerland showing the location of Lake Gerzensee in the Swiss foreland of
787 the Alps (triangle) and the bathymetry of the lake. Black circles indicate the fieldwork
788 measuring station (C1) and coring site

789

790 **Fig. 2** (a) Temperature and (b) dissolved oxygen concentrations ($[O_2]_{aq}$) measured at 1-m
791 depth intervals in the water column at the lake centre (location C1) over the fieldwork period
792 in 2012-2014. Colour figure online.

793

794 **Fig. 3** (a) $\delta^{13}C$ values of CO_2, particulate organic matter (POM), *Daphnia* for location C1
795 (pelagic zone) and *Daphnia* ephippia (whole lake) over the fieldwork period. (b) $\delta^{13}C$ values
796 of *Daphnia* ephippia, *Ceriodaphnia* ephippia, and *Plumatella* statoblasts (whole lake) over
797 the fieldwork period. *Daphnia* and flotsam $\delta^{13}C$ values represent single measurements
798 except for 25 February 2014 and 27 March 2014 when enough material was available for
799 three measurements of *Daphnia* $\delta^{13}C$. In those cases average values are presented

800

801 **Fig. 4** Age-depth model (black line, triangles) for core GER12 based on ^{210}Pb activity. The
802 grey line shows the accumulation of ^{137}Cs activity in the record (based on the ^{210}Pb-inferred
803 accumulation rate), with the diamond showing the location of the lower activity peak of ^{137}Cs
804 in the ^{210}Pb-based age model. This peak is correlated with atmospheric nuclear bomb testing
805 in AD 1963 and coincides with the only measurable ^{241}Am activity in the core (1.9±0.2 Bq/kg).

806

807 **Fig. 5** $\delta^{13}C$ values of fossil invertebrate remains of *Daphnia*, *Ceriodaphnia* and *Plumatella* in
808 sediment core GER12 and from the flotsam of the lake (flotsam values plotted above 0 cm
809 sediment core depth). Symbols in the downcore record represent the average depth of the

measurements in cases for which samples were pooled to obtain sufficient weight for $\delta^{13}C$ analysis. Measurements may represent remains from up to 6 cm of sediment (see text for details.

Figures

Figure 1

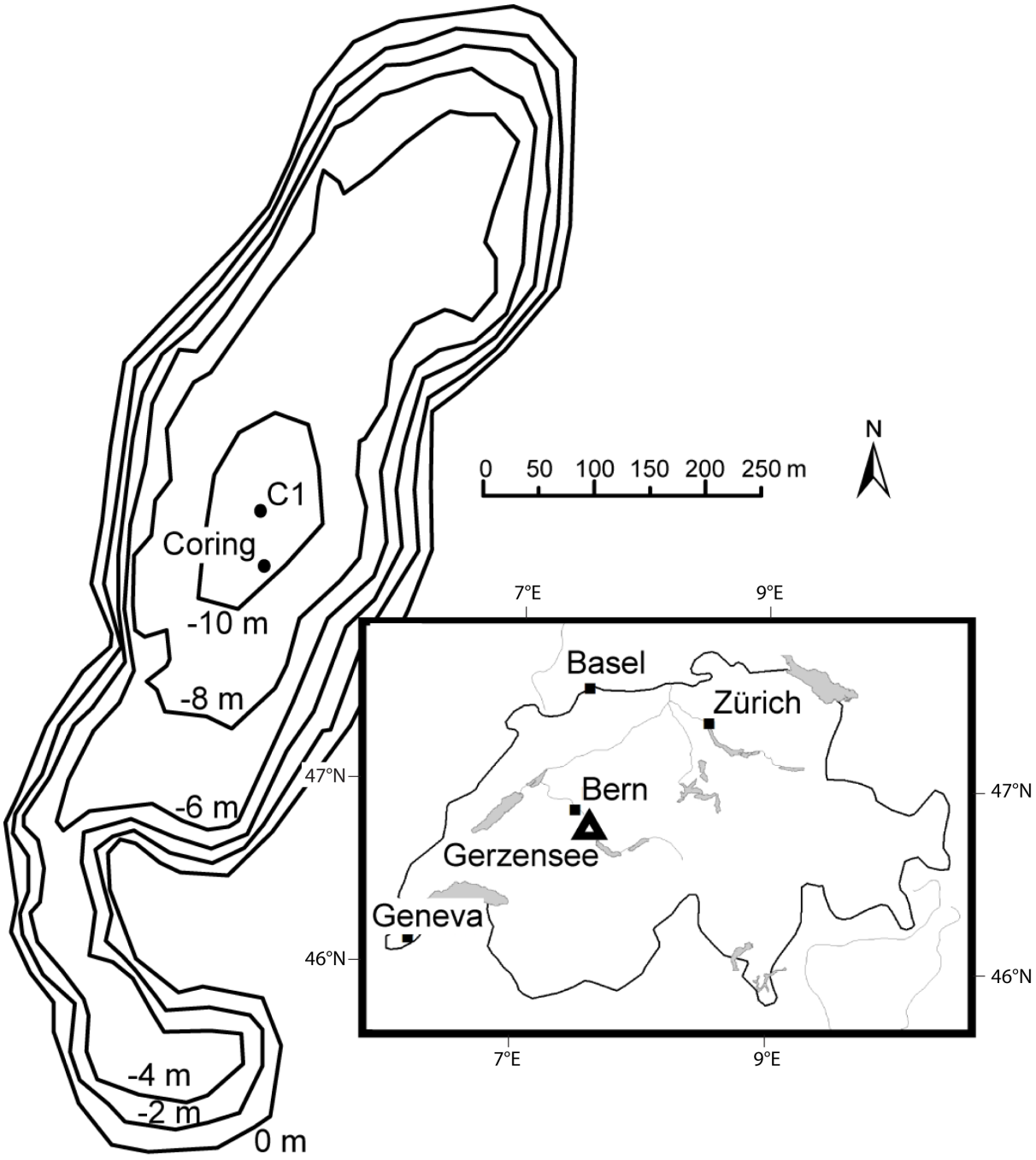
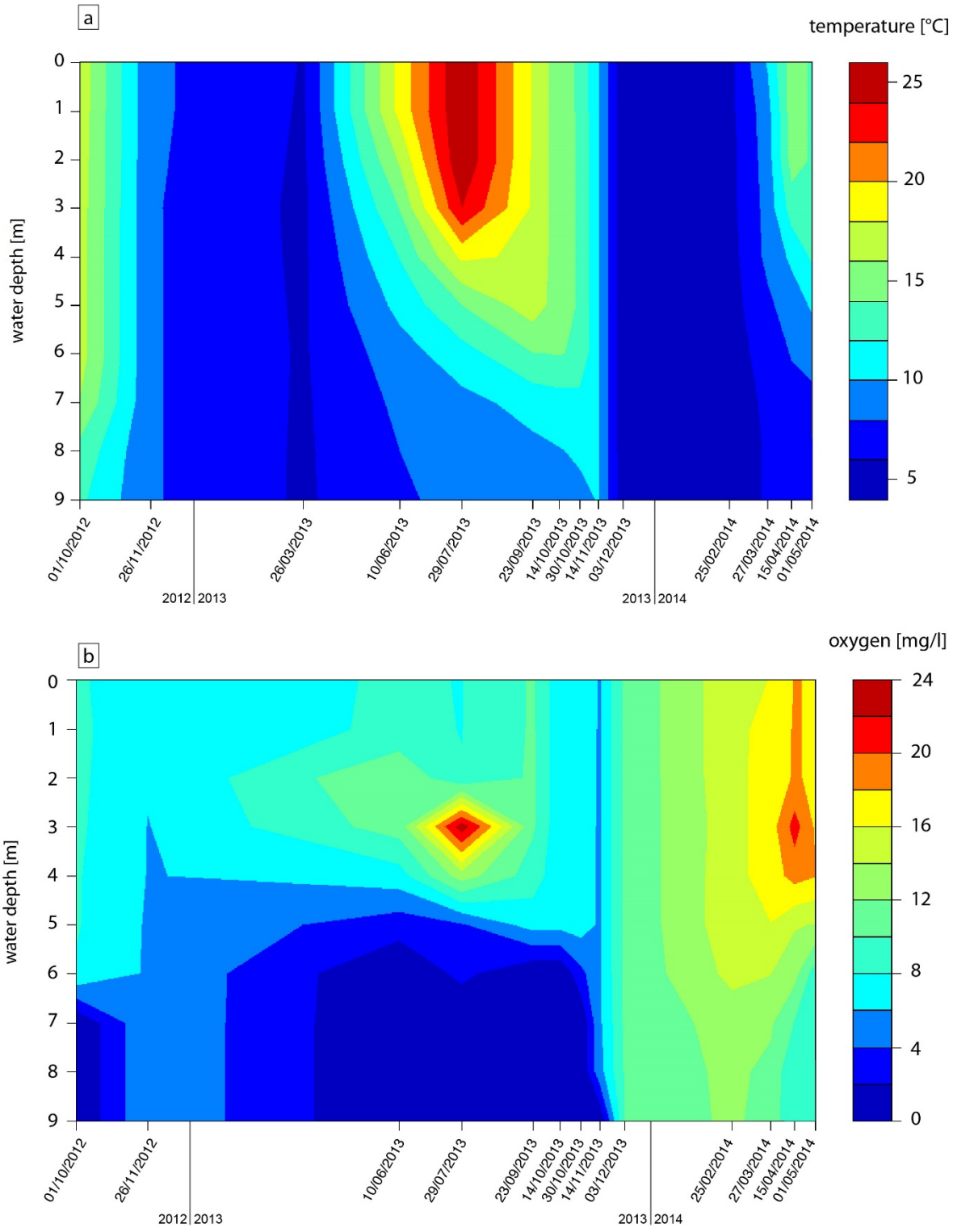
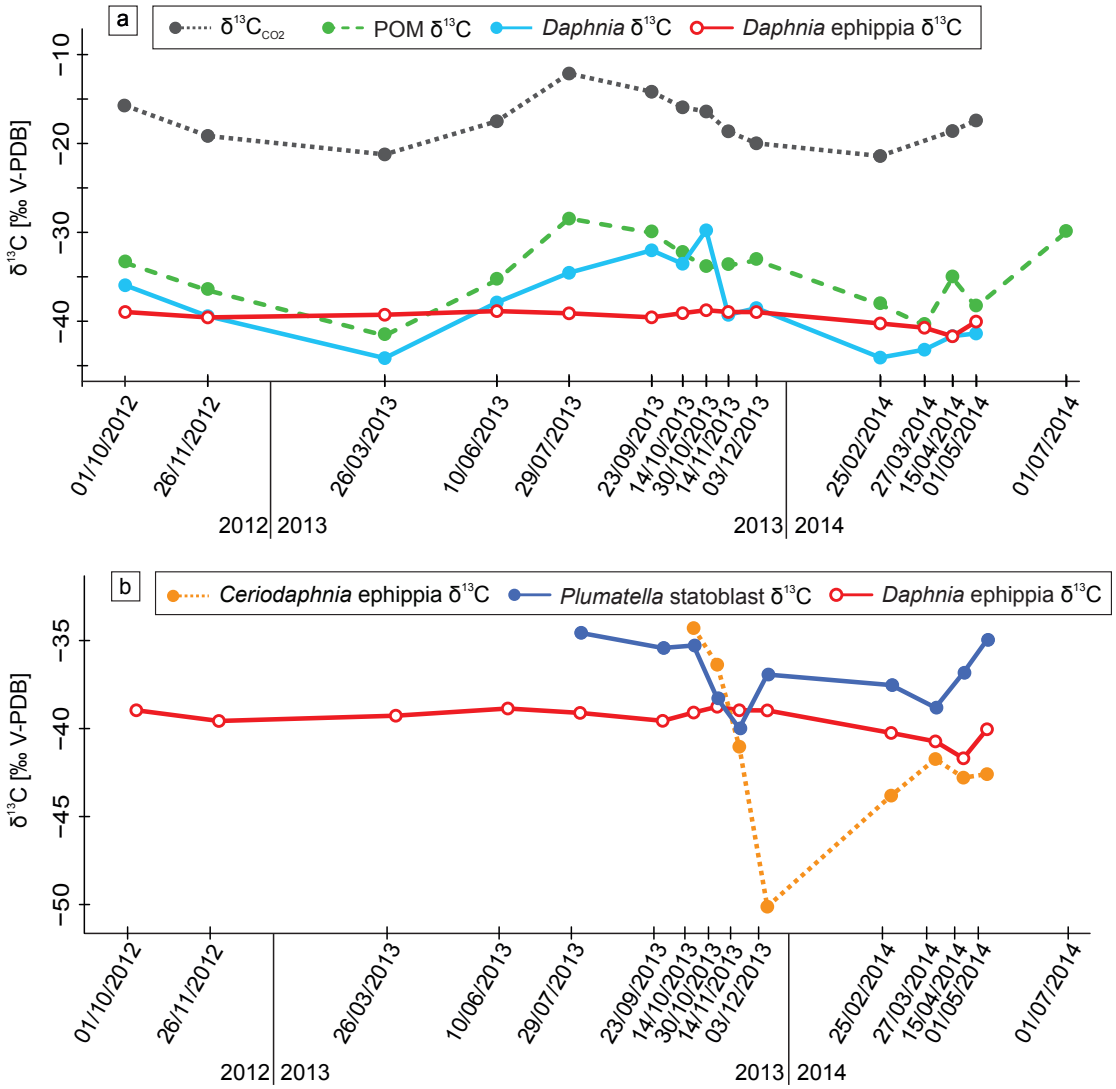


Figure 2


a
temperature [°C]
water depth [m]
0
1
2
3
4
5
6
7
8
9
25
20
15
10
5
01/10/2012
26/11/2012
26/03/2013
10/06/2013
29/07/2013
23/09/2013
14/10/2013
30/10/2013
14/11/2013
03/12/2013
25/02/2014
27/03/2014
15/04/2014
01/05/2014
2012
2013
2013
2014
b
oxygen [mg/l]
water depth [m]
24
20
16
12
8
4
0

820

821 Figure 3

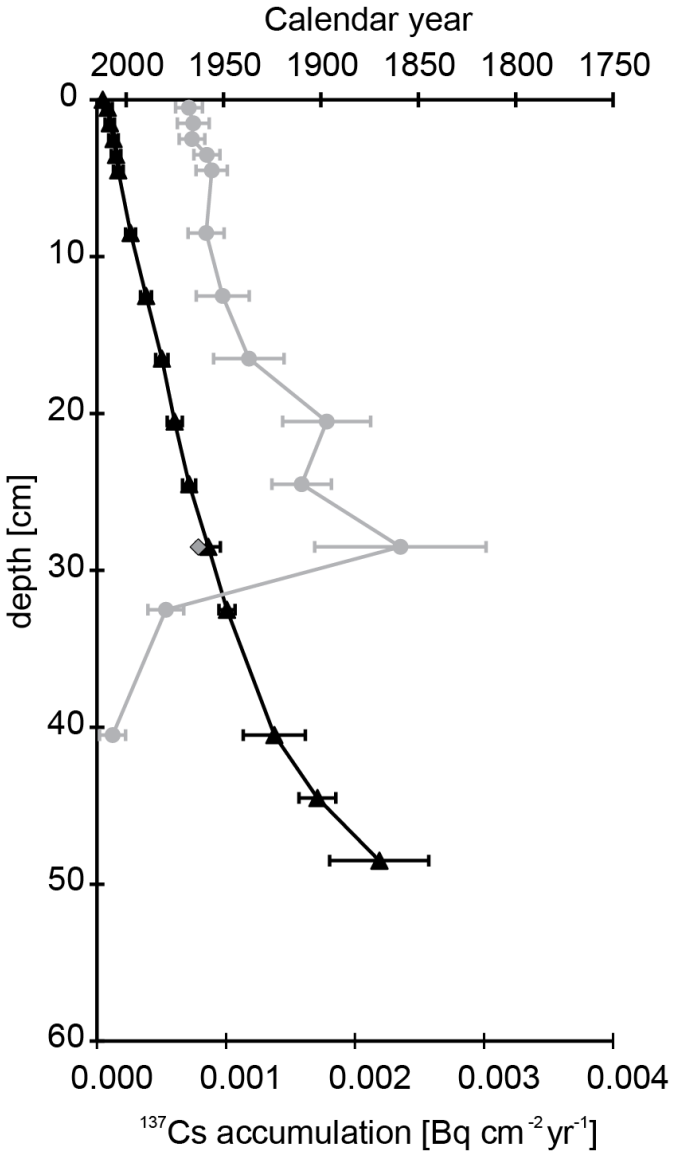


822

823

823

824 Figure 4

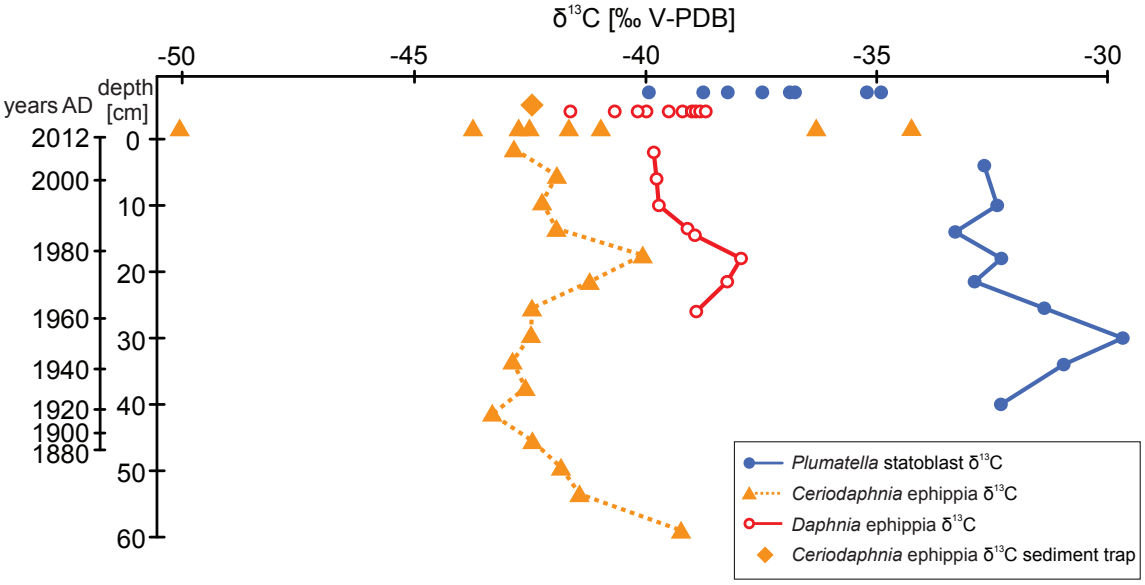


825

826

826

827 Figure 5



828

829

Electronic Supplementary Material

Figure Captions

Fig. S1

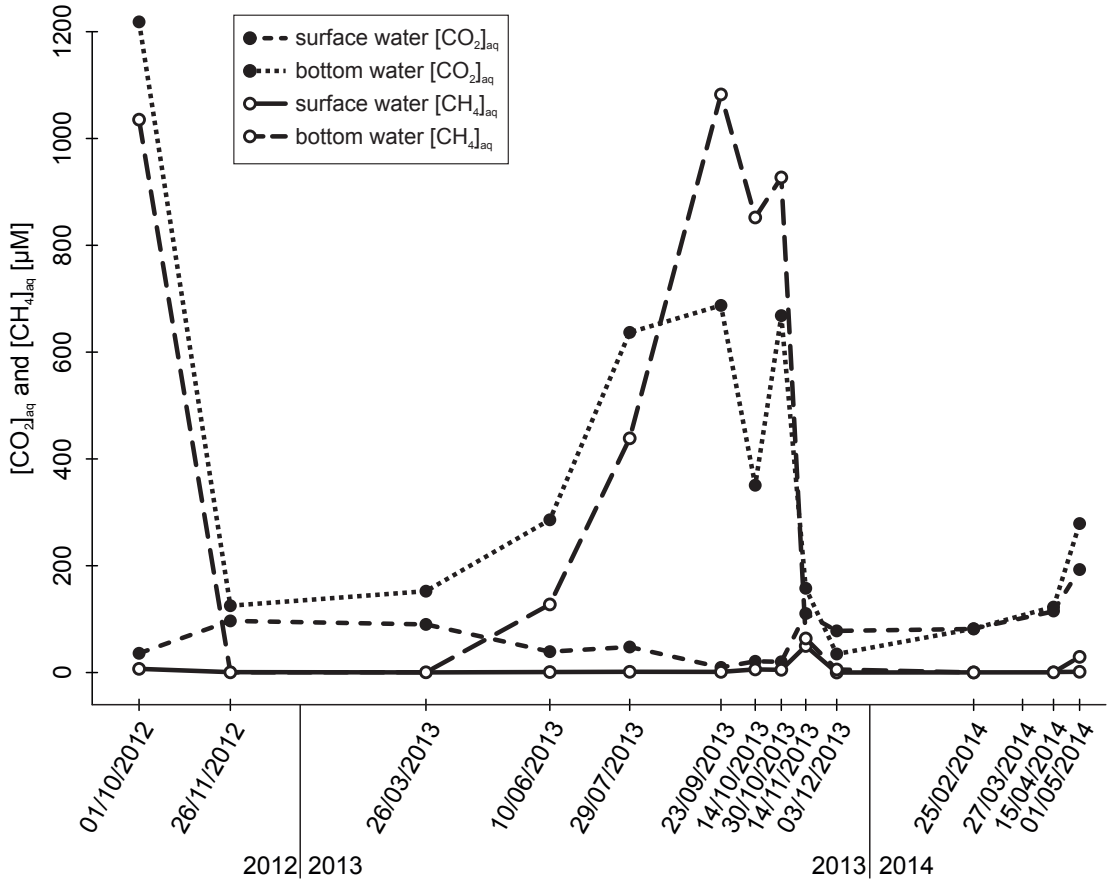
Dissolved CO_2 ($[CO_2]_{aq}$) and CH_4 ($[CH_4]_{aq}$) concentrations for surface (0.7 m water depth) and bottom waters (8 m water depth) at the lake centre (location C1) over the fieldwork period in 2012-2014

Fig. S2

(a) Relationship between surface water chlorophyll *a* concentrations (0.7 m water depth) and *Daphnia* $\delta^{13}C$ at the lake centre (location C1); (b) relationship between log-transformed bottom water dissolved CH_4 concentrations ($[CH_4]_{aq}$) (8 m water depth) and *Daphnia* $\delta^{13}C$ at the lake centre (location C1); (c) relationship of the difference between particulate organic matter (POM) $\delta^{13}C$ and *Daphnia* $\delta^{13}C$ in the surface water ($\Delta^{13}C_{POM\text{-}Daph}$) and bottom water (8 m water depth) $[CH_4]_{aq}$; (d) surface (0.7 m water depth) and bottom water (8 m water depth) POM $\delta^{13}C$ at the lake centre (location C1)

847

848 Figure S1

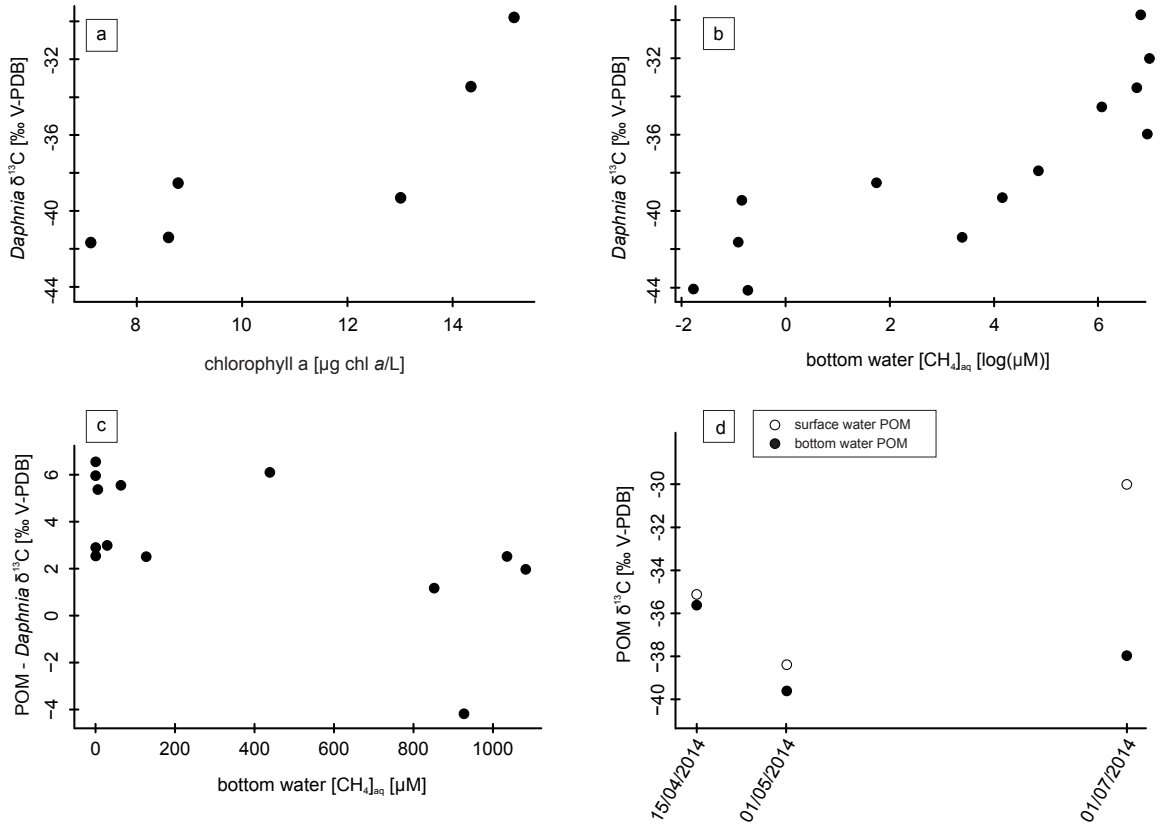


849

850

850

851 Figure S2



852

853

Table S1 Samples collected and analysed in the course of repeated fieldwork on Gerzensee between October 2012 and July 2014. "x" denotes that a sample was collected, "-" shows where no sample was collected

Fieldwork dates [d.m.yr]	Temp	$[O_2]_{aq}$	*Daphnia* $\delta^{13}C$	*Daphnia* ephippia $\delta^{13}C$	*Ceriodaphnia* ephippia $\delta^{13}C$	*Plumatella* statoblast $\delta^{13}C$	DIC	$\delta^{13}C_{POM}$ (0.7 m)	$\delta^{13}C_{POM}$ (7 m)	Chl *a* [µg/l]	$[CH_4]$ & $[CO_2]$
01.-03.10.2012	x	x	x	x	-	-	x	x	-	-	x
26.-28.11.2012	x	x	x	x	-	-	x	x	-	-	x
26.-28.03.2013	x	x	x	x	-	-	x	x	-	-	x
10.-13.06.2013	x	x	x	x	-	-	x	x	-	-	x
29.-31.07.2013	x	x	x	x	-	x	x	x	-	-	x
23.-25.09.2013	x	x	x	x	x	x	x	x	-	-	x
14.10.2013	x	x	x	x	x	x	x	x	-	x	x
30.10.2013	x	x	x	x	x	x	x	x	-	x	x
14.11.2013	x	x	x	x	x	x	x	x	-	x	x
03.12.2013	x	x	x	x	x	x	x	x	-	x	x
25.02.2014	x	x	x	x	x	x	x	x	-	-	x
26.03.2014	x	-	-	x	x	x	-	-	-	-	x
15.04.2014	x	x	x	x	x	x	x	x	x	x	x